

FIG. 1

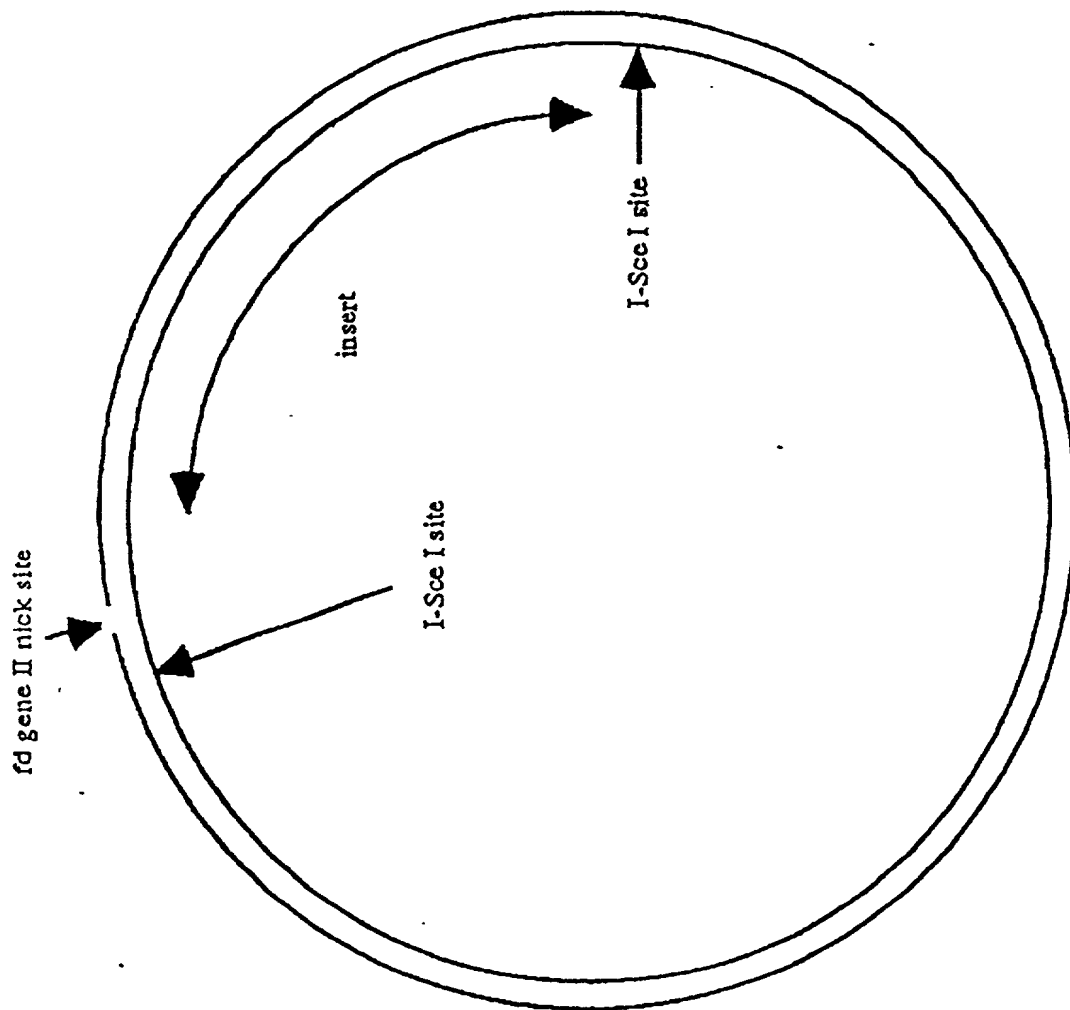


FIG. 2

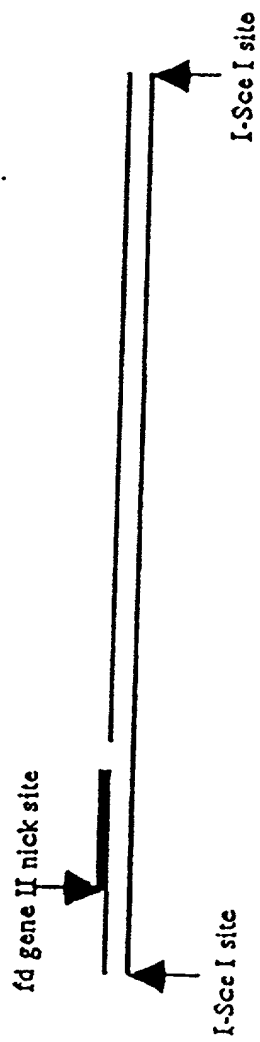
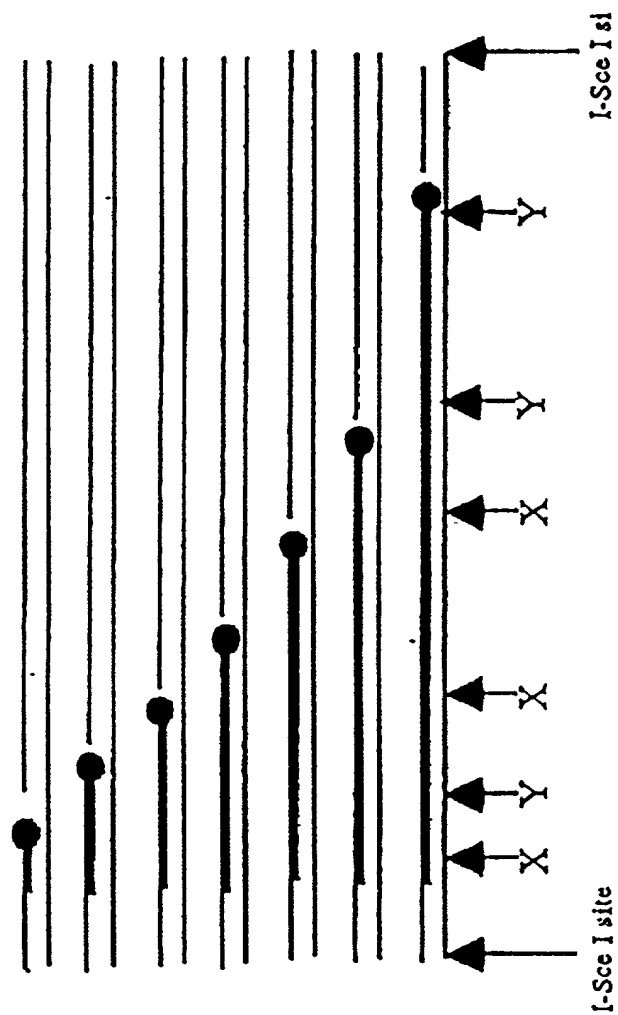


FIG. 3



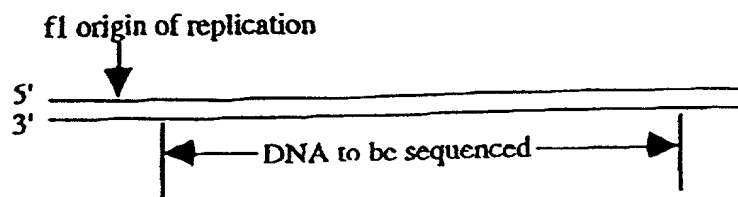


FIG. 4A



FIG. 4B



FIG. 4C



FIG. 4D



FIG. 4E

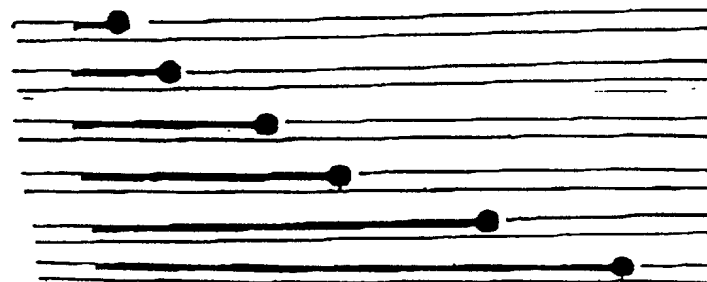
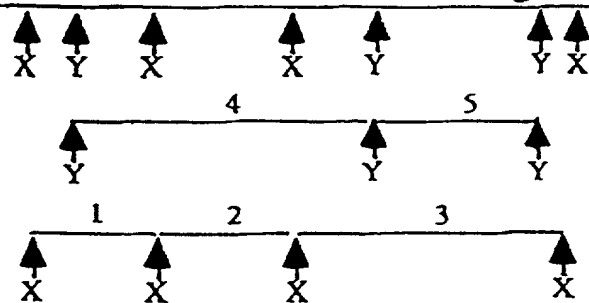


FIG. 4F



FIG. 4G



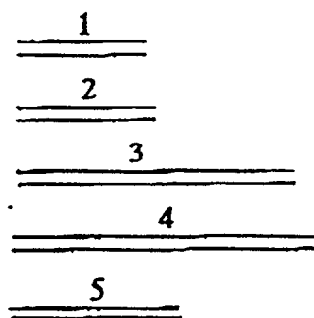


FIG. 4H

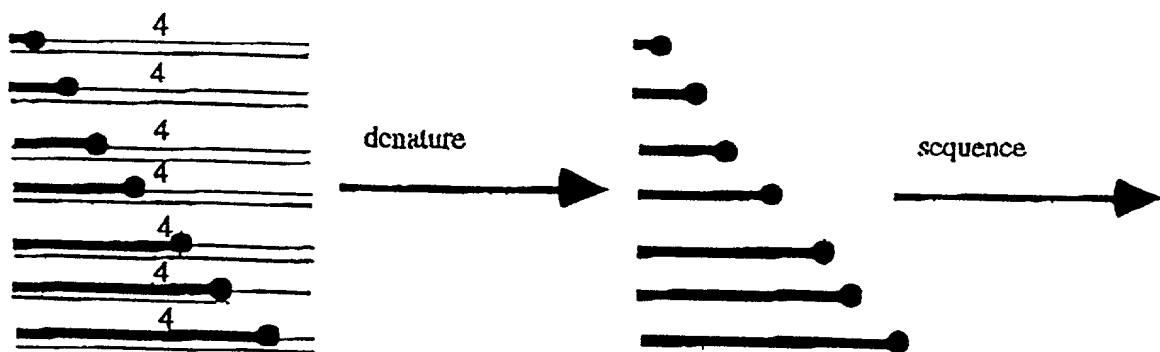
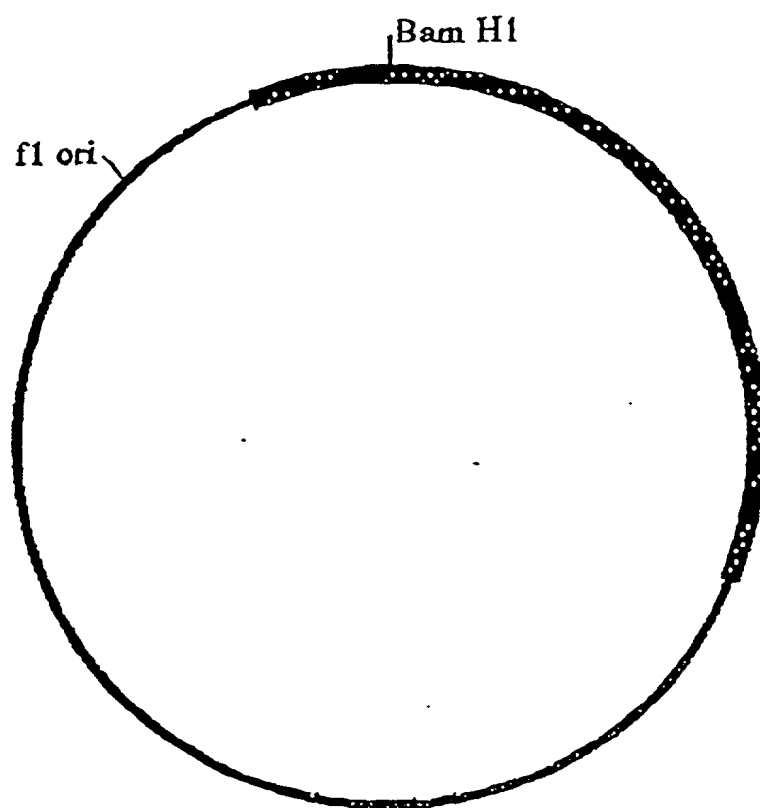


FIG. 4I

FIG. 5



Restriction with Bam H1
to linearize SRR products

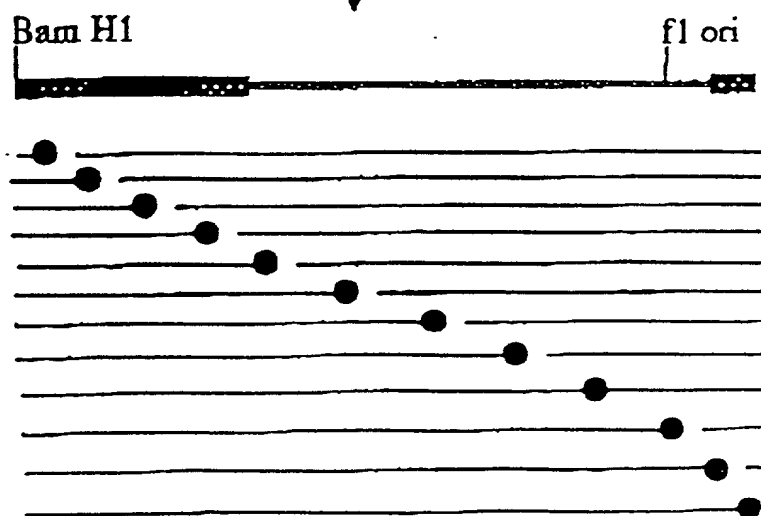
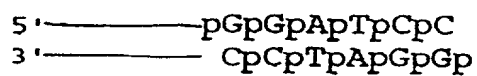
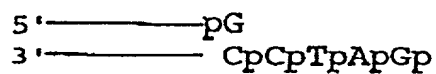


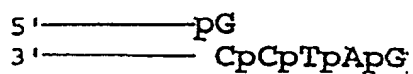
FIG. 6



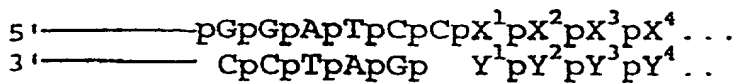
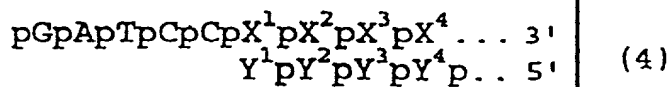
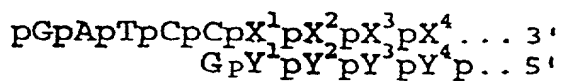
(1)
↓



(2)
↓



(3)
↓



↓

STRAND REPLACEMENT REACTION

FIG. 7A

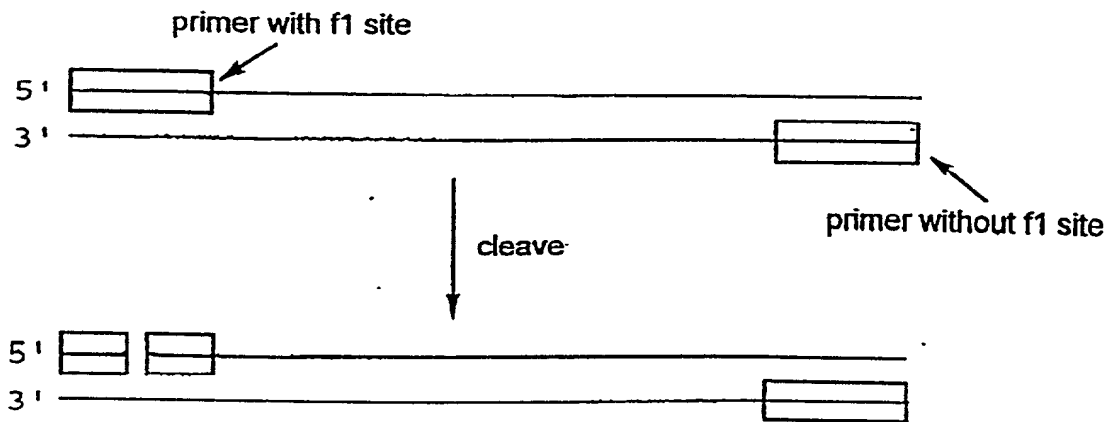


FIG. 7B

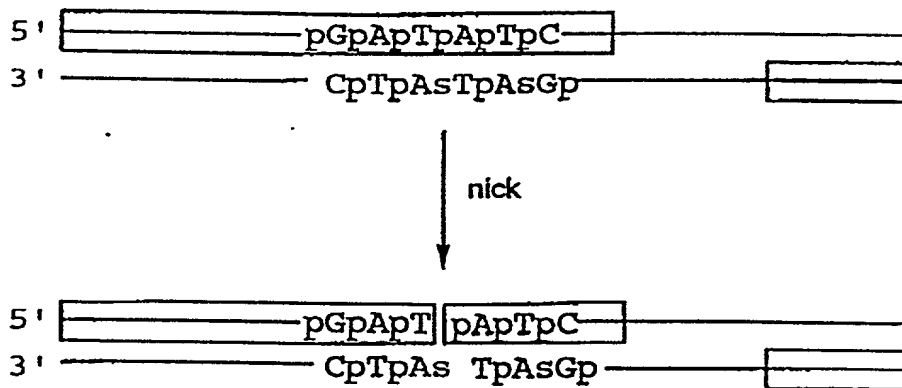


FIG. 7C

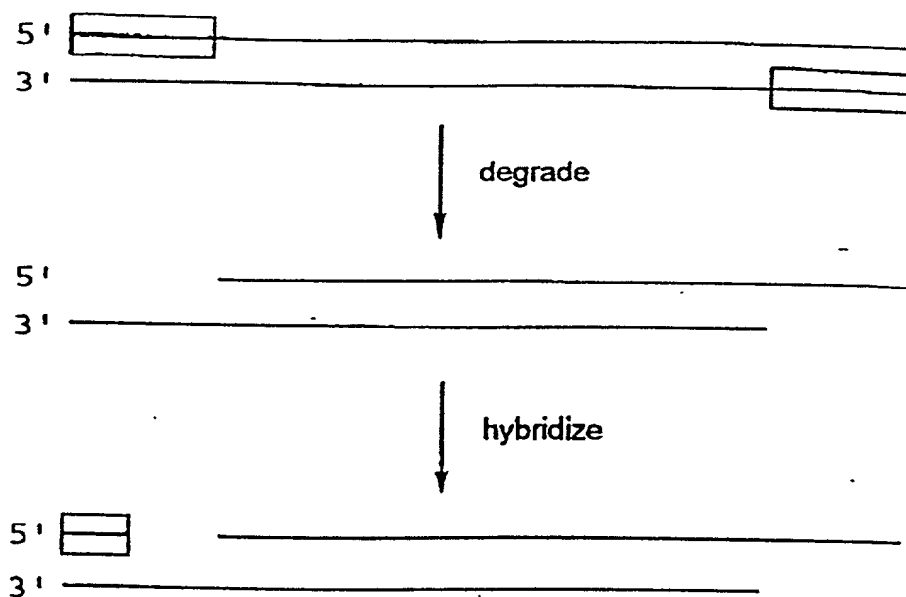
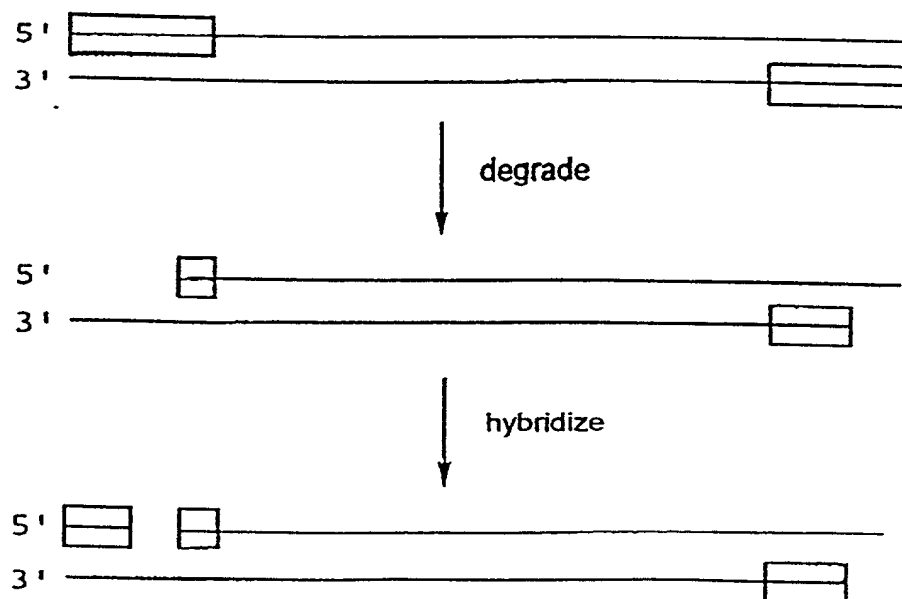


FIG. 7D



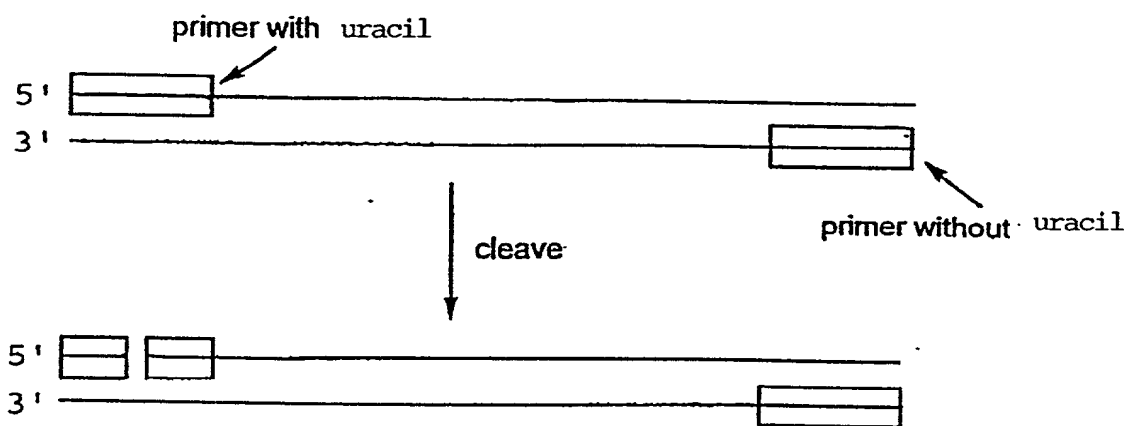


FIG. 7E

FIG. 8

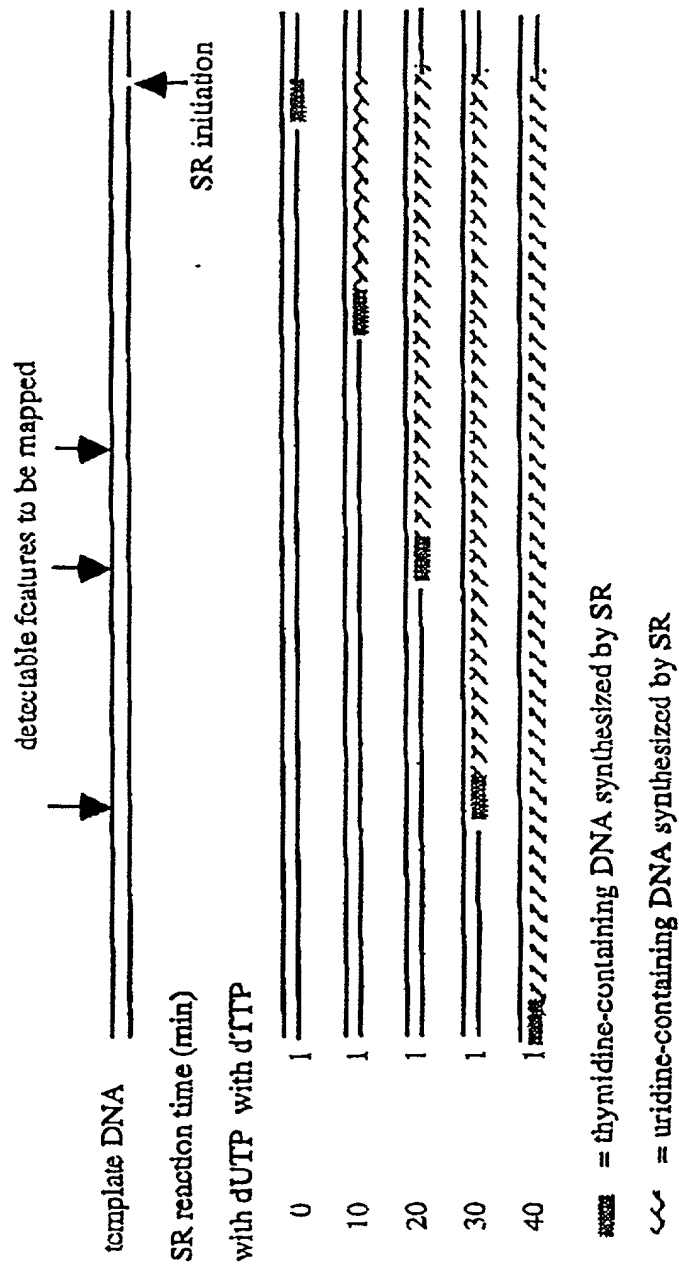


FIG. 9

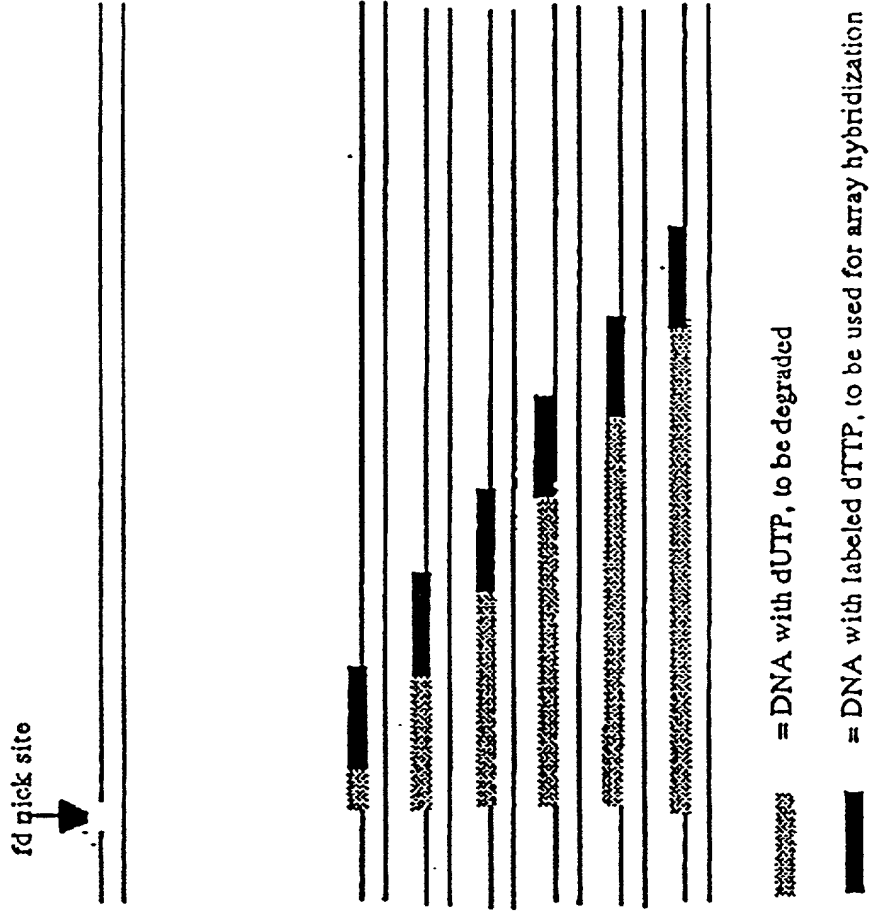


FIG. 10A

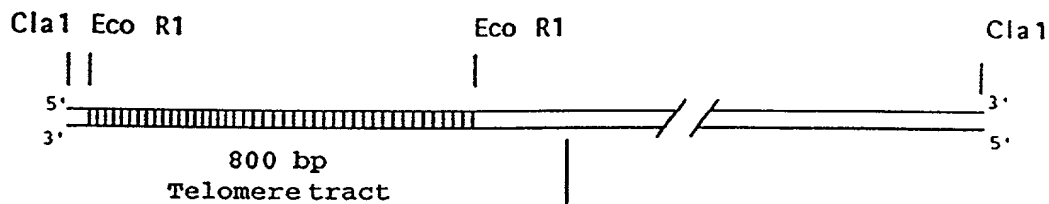


FIG. 10B

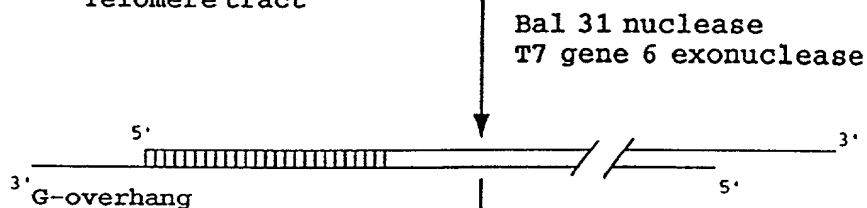


FIG. 10C

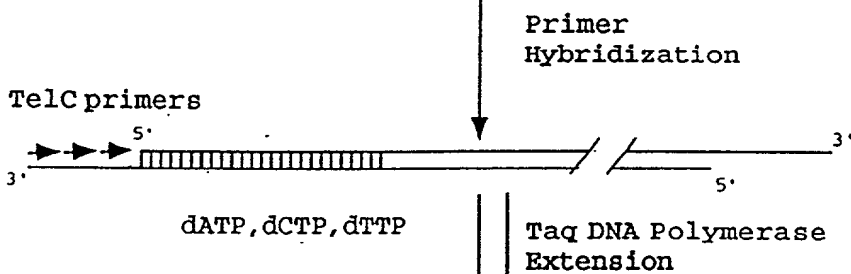


FIG. 10D

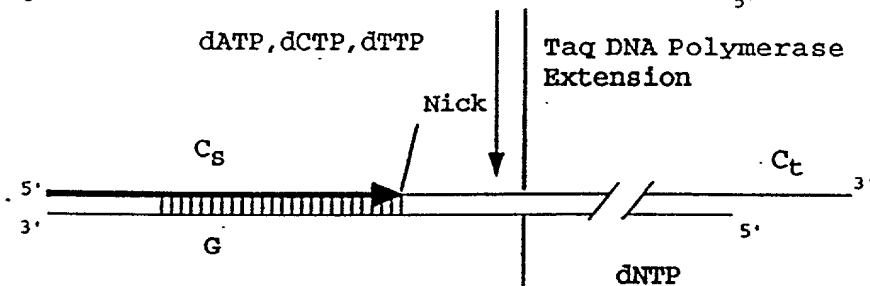


FIG. 10E

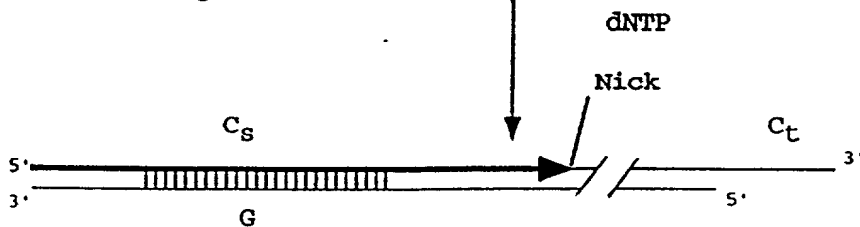


FIG. 11

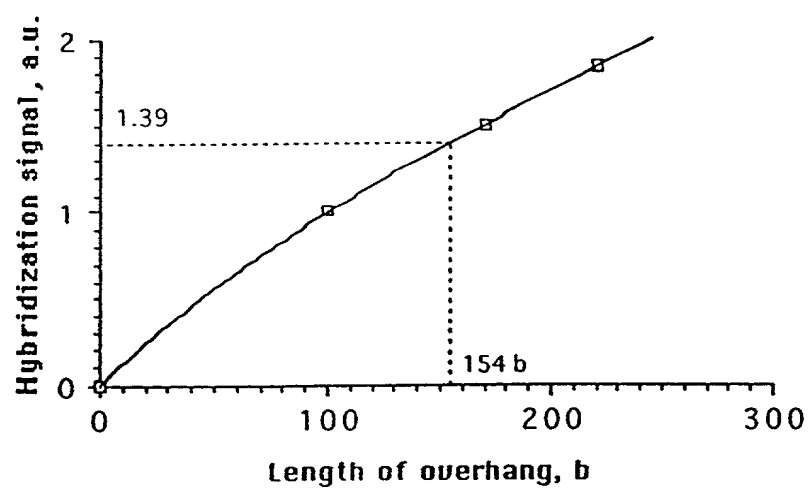
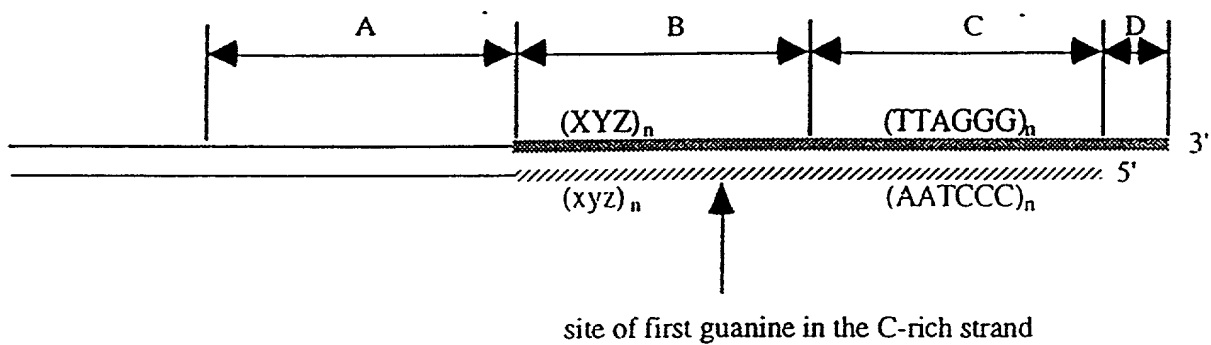


FIG. 12



reaction time (min)

10

////////////////////

20

////////////////////////////////////

30

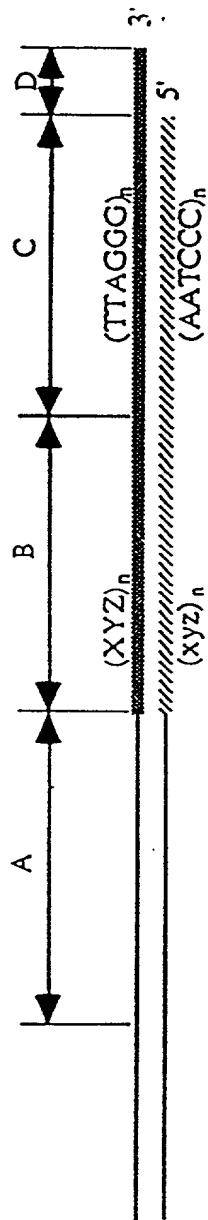
////////////////////////////////////

40

////////////////////////////////////

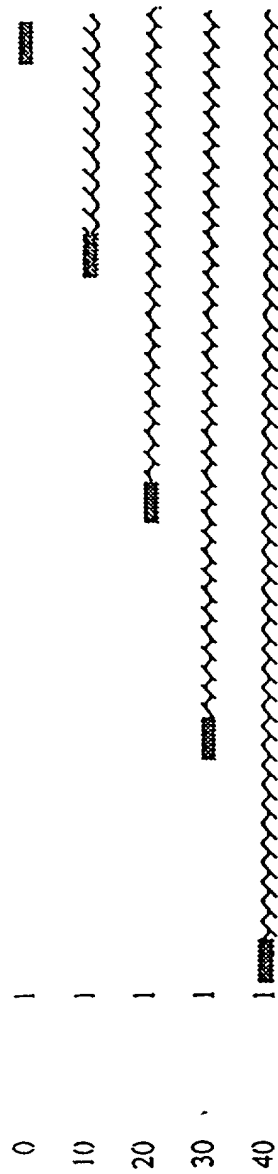
//// = DNA synthesized by PENT using only dATP, dTTP, and dCTP

FIG. 13



reaction time (min)

with dUTP with dTTP



===== = thymine-containing DNA synthesized by PENT

----- = uridine-containing DNA synthesized by PENT

FIG. 14A

FIG. 14A

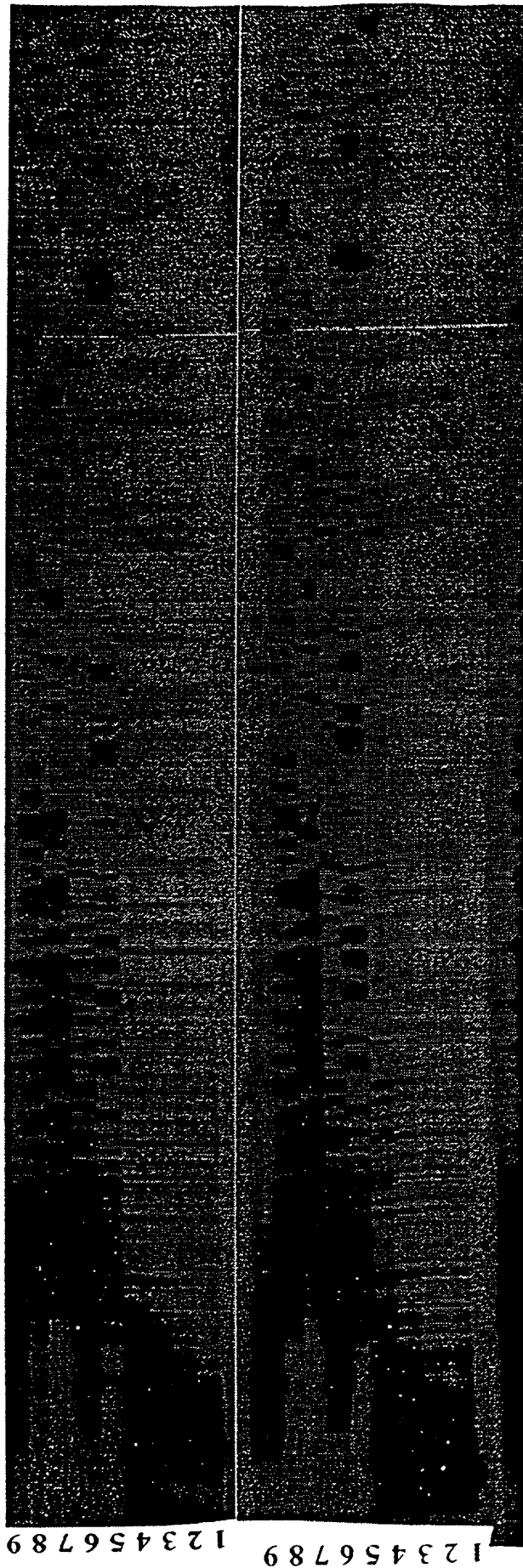


FIG. 14B

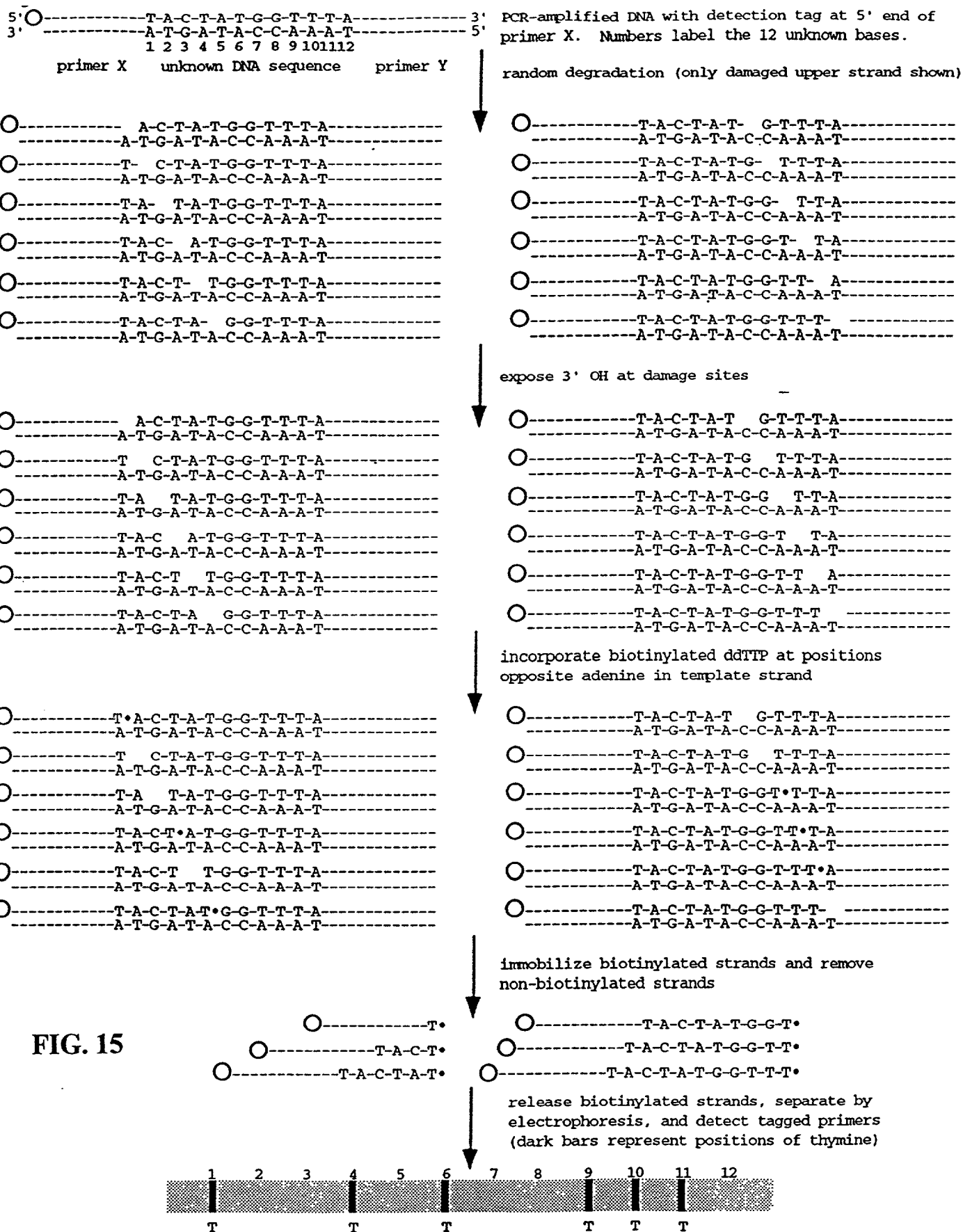
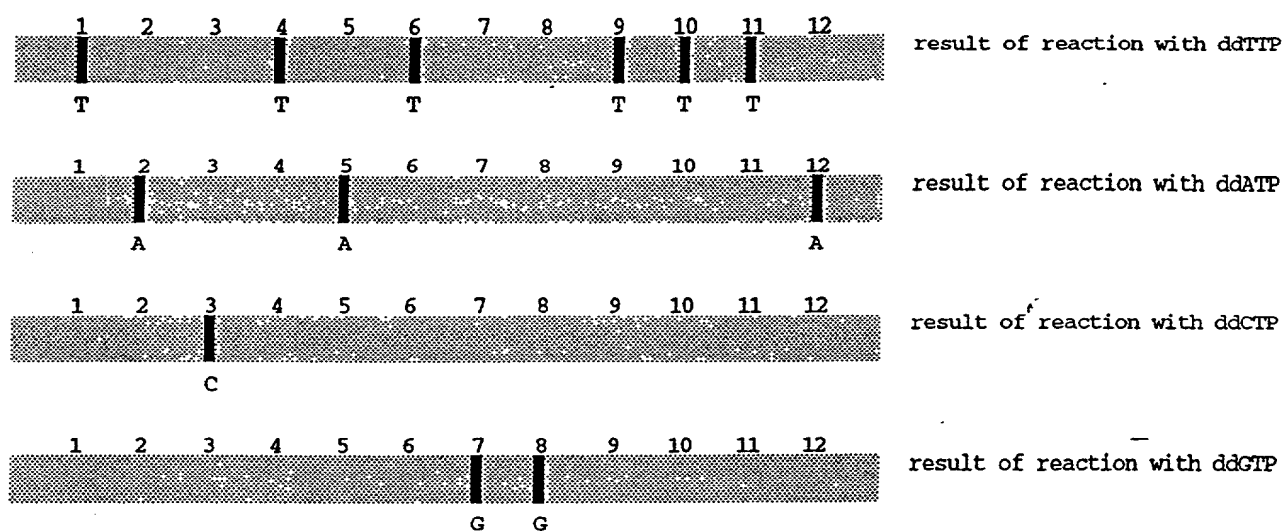


FIG. 15

Results of size separation of detectable products of four ddNTP reactions



summation of ddNTP results into complete base sequence



FIG. 16

5' O-----T-A-C-T-A-T-G-G-T-T-T-A-----3'
 3'-----A-T-G-A-T-A-C-C-A-A-A-T-----5'
 1 2 3 4 5 6 7 8 9 10 11 12

primer X unknown DNA sequence primer Y

PCR-amplified DNA with detection tag at 5' end of primer X. Numbers label the 12 unknown bases.

random degradation (only damaged upper strand shown)

O-----A-C-T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T- C-T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A- T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C- A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T- T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A- G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

O-----T-A-C-T-A-T- G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G- T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G- T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T- T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T- A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T-T-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

expose 3' OH at damage sites

O-----A-C-T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T C-T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

O-----T-A-C-T-A-T G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T-T-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

incorporate biotinylated ddTTP at positions opposite adenine in template strand

O-----T•A-C-T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T C-T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A T-A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T•A-T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T T-G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T•G-G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

O-----T-A-C-T-A-T G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T•T•A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T•T•A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T-T•A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T-T-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

immobilize biotinylated strands and remove non-biotinylated strands

O-----T-A-C-T-A-T-G-G-T•T•T•
 O-----T-A-C-T-A-T-G-G-T•T•T•
 O-----T-A-C-T-A-T-G-G-T-T•T•

release biotinylated strands, separate by electrophoresis, and detect tagged primers (dark bars represent positions of terminal thymine)

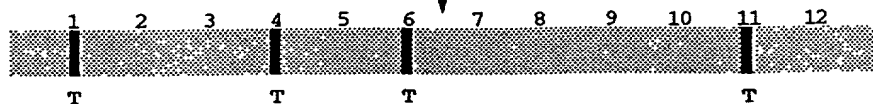
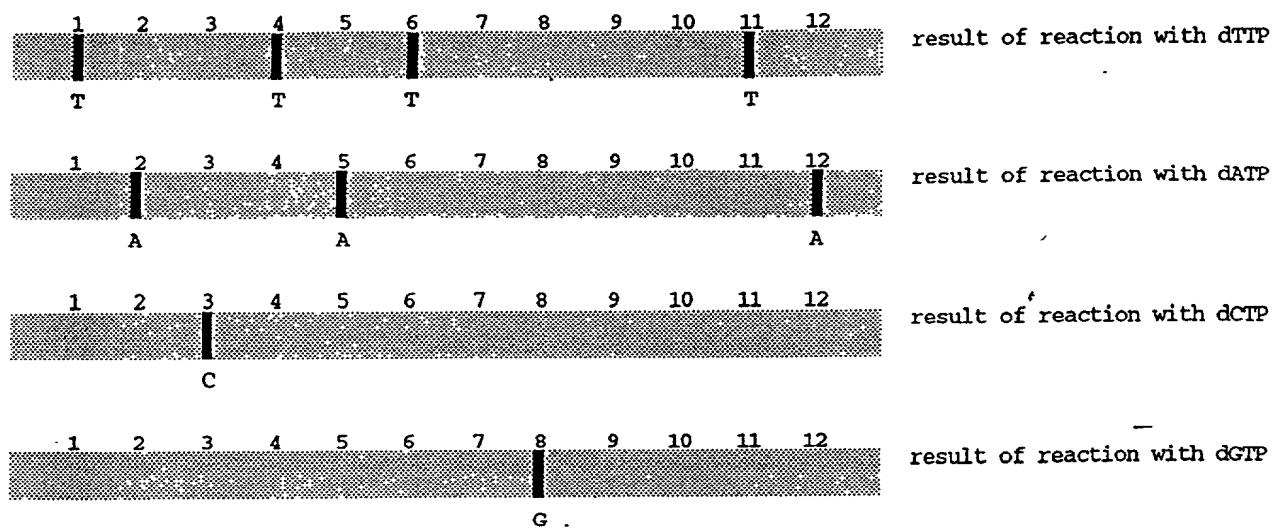


FIG. 17

Results of size separation of detectable products of four dNTP reactions



Summation of dNTP results into complete base sequence
(positions of bases in parentheses are inferred)

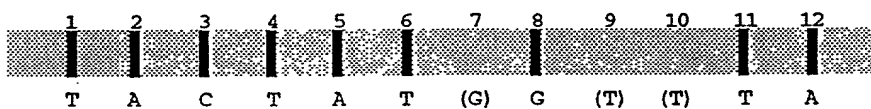


FIG. 18

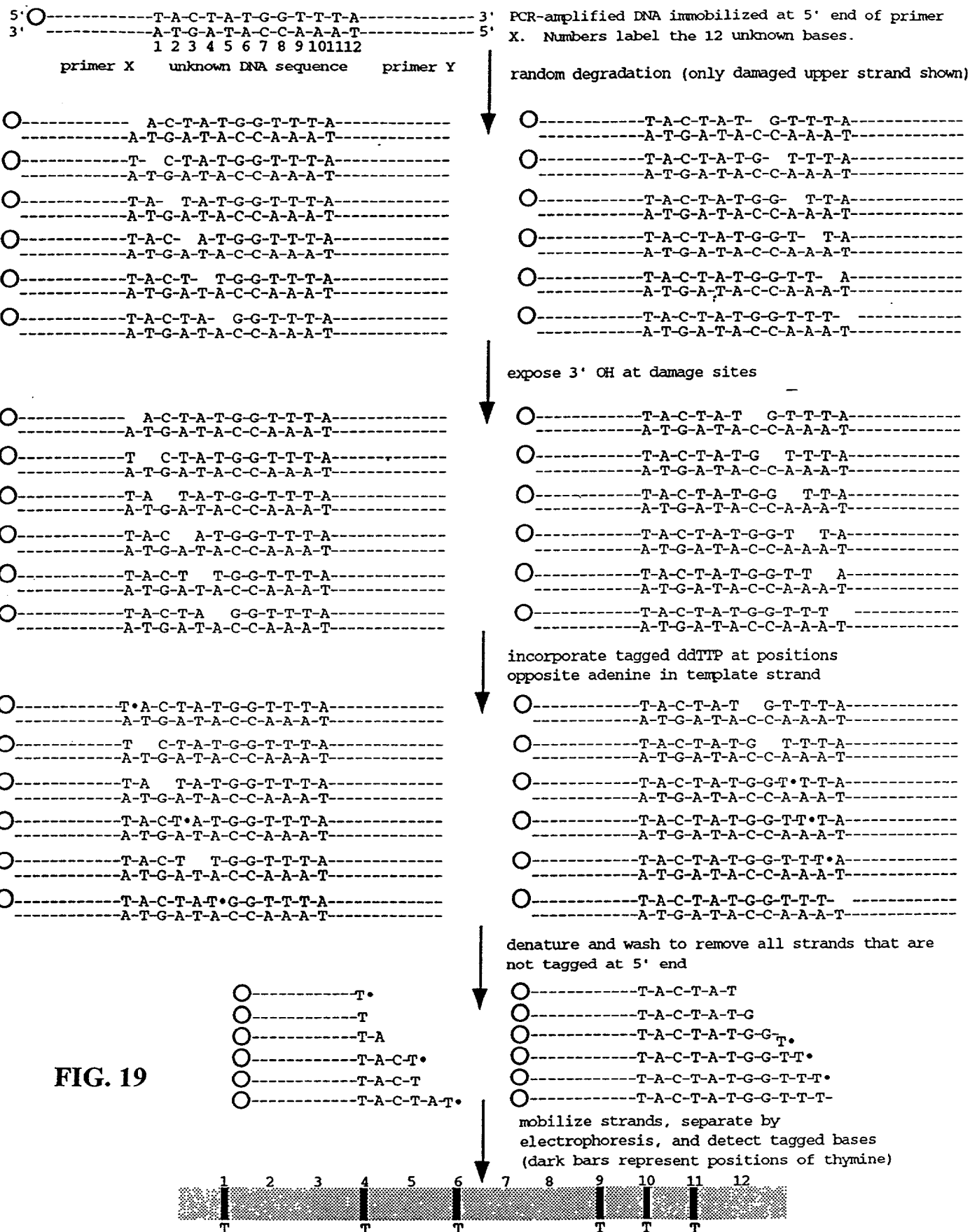
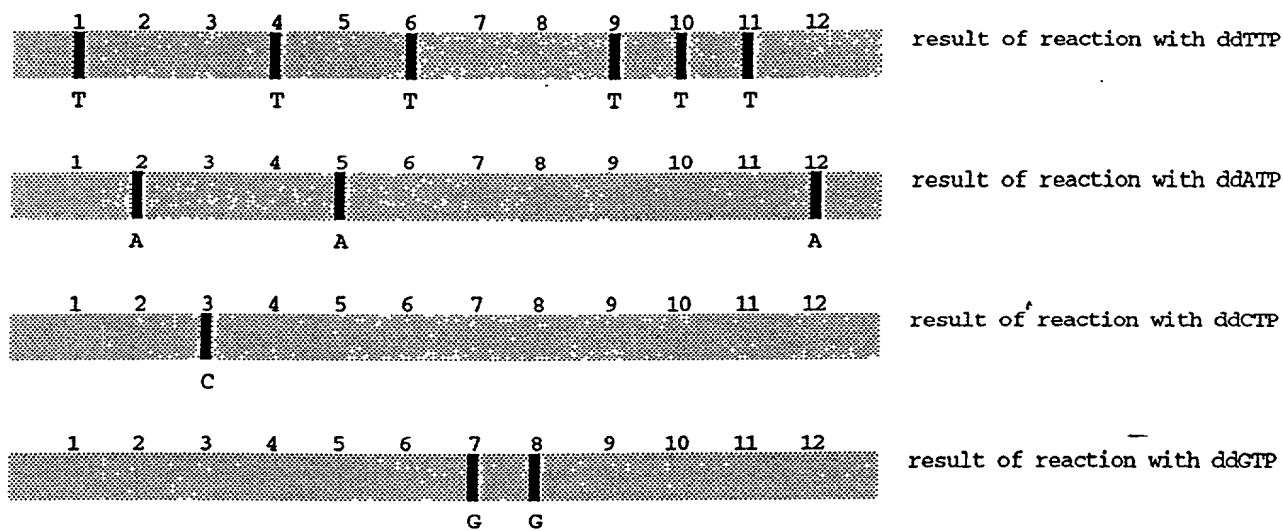


FIG. 19

Results of size separation of detectable products of four ddNTP reactions



summation of ddNTP results into complete base sequence

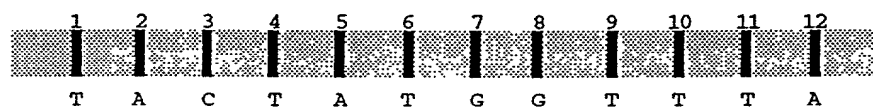
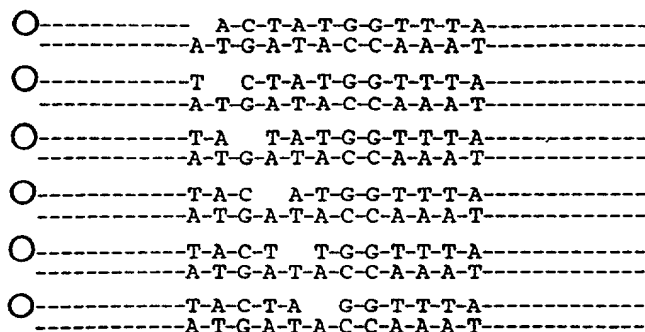
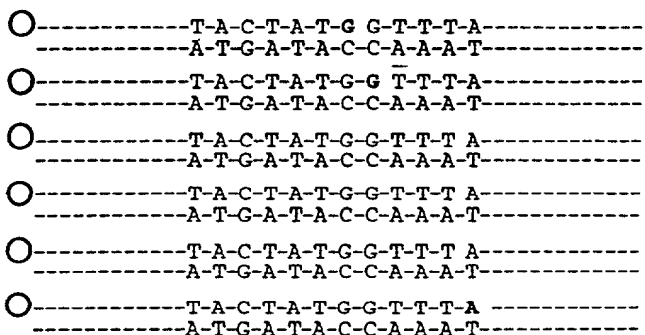


FIG. 20

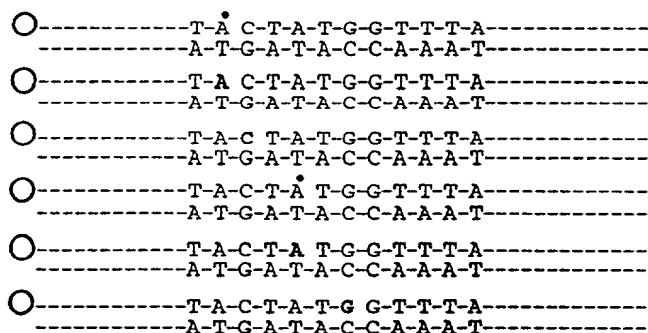
PCR amplify, immobilize, and expose 3' OH
at random sites as in Fig. 5.



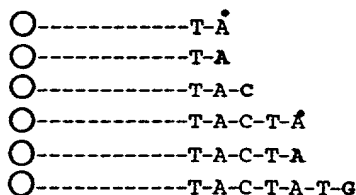
Block ends opposite T,G' & C with ddATP,ddGTP,ddCTP
(shown in bold letters), remove ddNTPs, then add dTTP.



Block ends opposite A,G & C with ddTTP,ddGTP,ddCTP
(shown in bold letters), remove ddNTPs, then add
tagged ddATP.



Denature and wash to remove all strands that are
not tagged at 5' end.



Mobilize strands, separate by electrophoresis,
and detect tagged bases (dark bars).

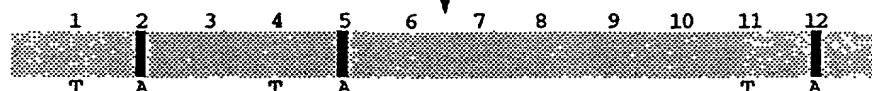
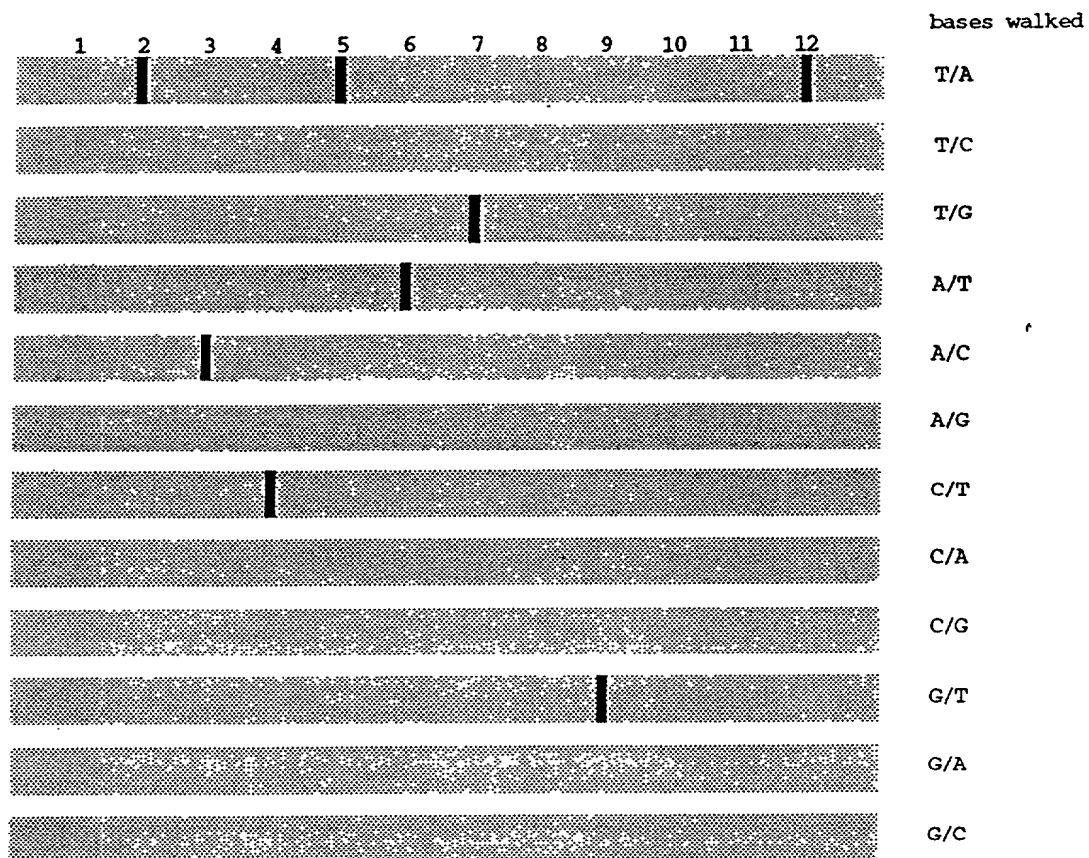


FIG. 21

Size separation of the products of twelve 2-base walk reactions



Assembly of complete sequence from the results of individual reactions
(inferred bases in parentheses)



FIG. 22

PCR amplify, immobilize, and expose 3' OH at random sites as in Fig. 5.

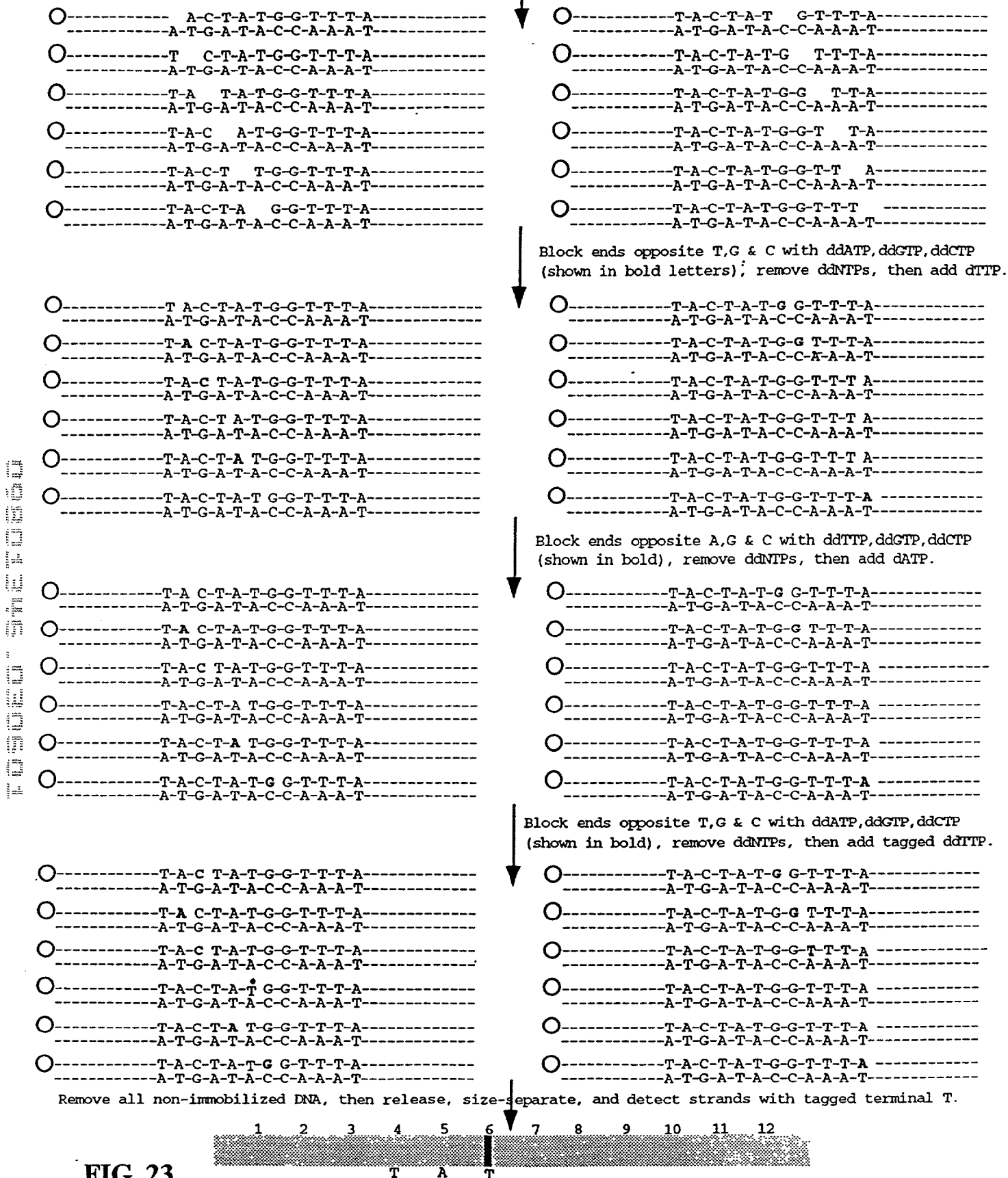


FIG. 23

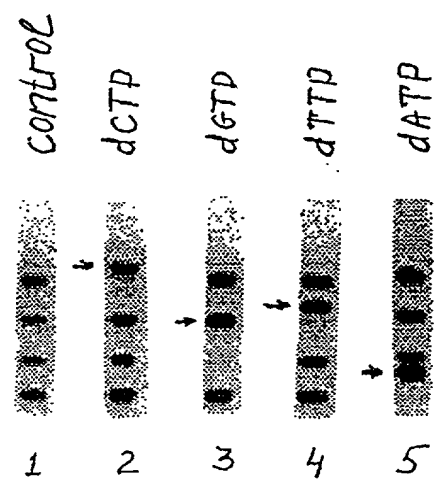


FIG. 24

dd(-A) dd(-T) dd(-G) dd(-C)

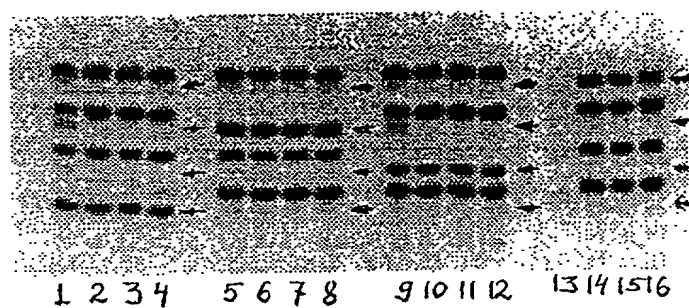


FIG. 25

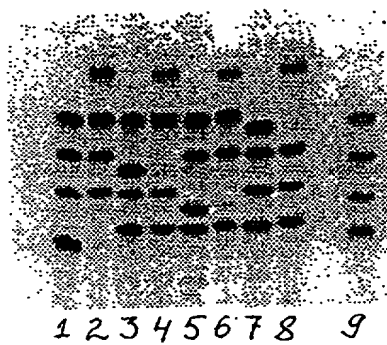


FIG. 26

Fe/EDTA DNase I

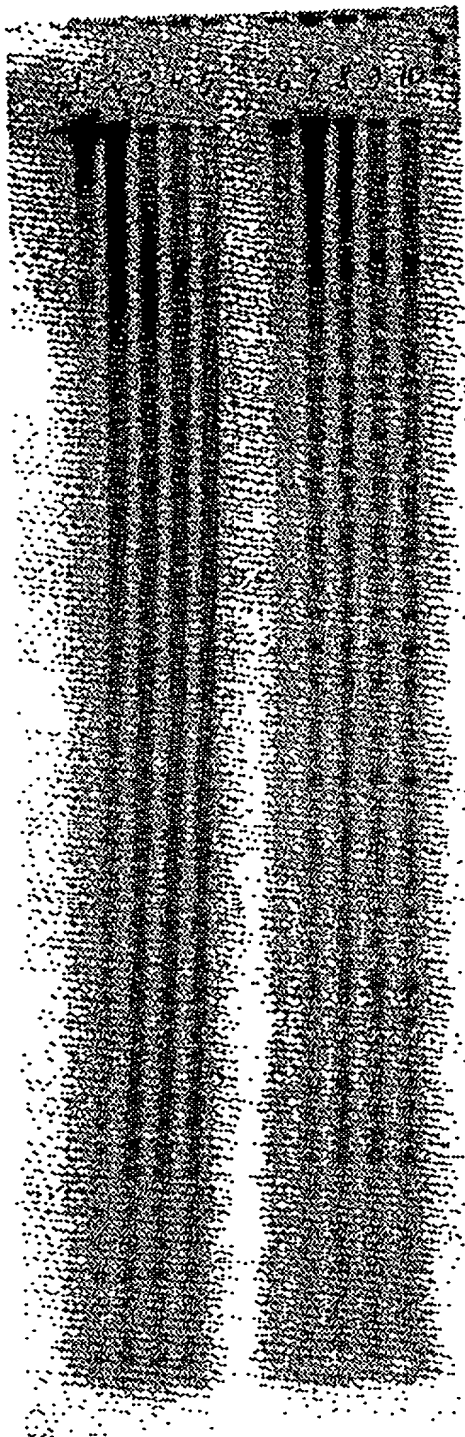
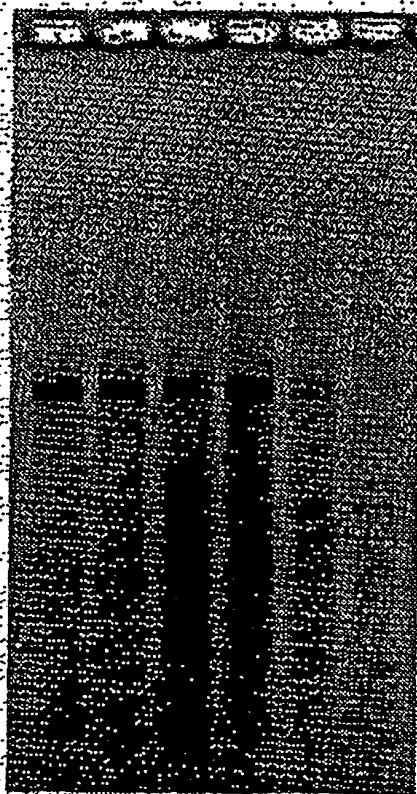


FIG. 27

EtBr staining

1 2 3 4 5 6



32 P-ATP

7 8 9 10 11 12

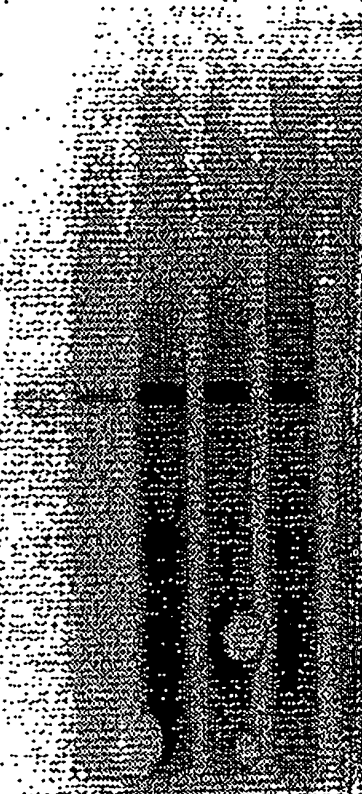


FIG. 28A

FIG. 28B

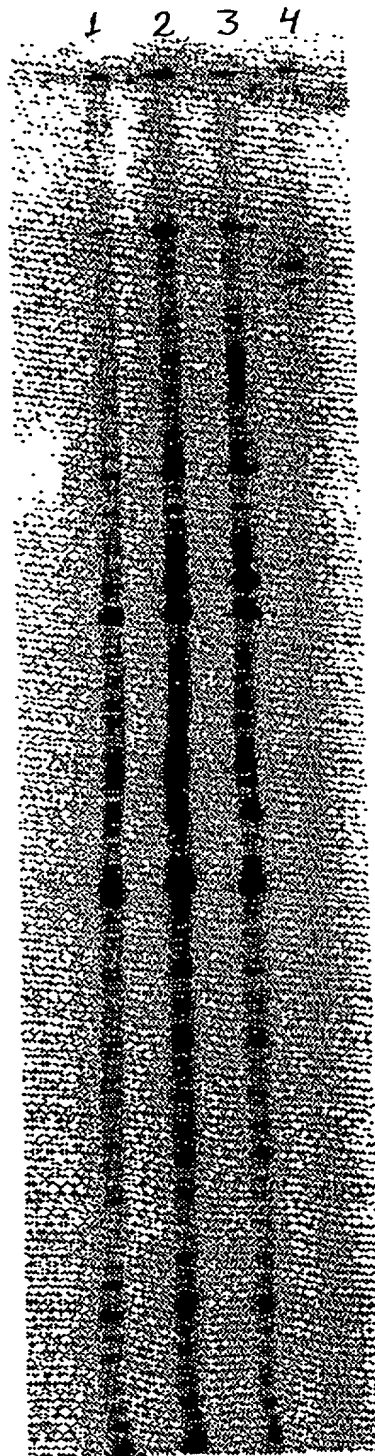
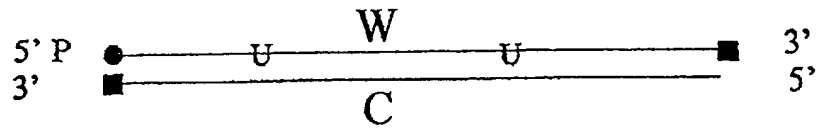


FIG. 29

FIG. 30A



● - 5' -phosphate

■ - 3' dideoxynucleotide or NH₃ group

5'	_____	X	3' OH	4 C-X oligos
5'	_____	XY	3' OH	16 C-XY oligos
5'	_____	XYZ	3' OH	64 C-XYZ oligos

X, Y and Z are A, T, G or C

FIG. 30B

Map of the XYZ sites

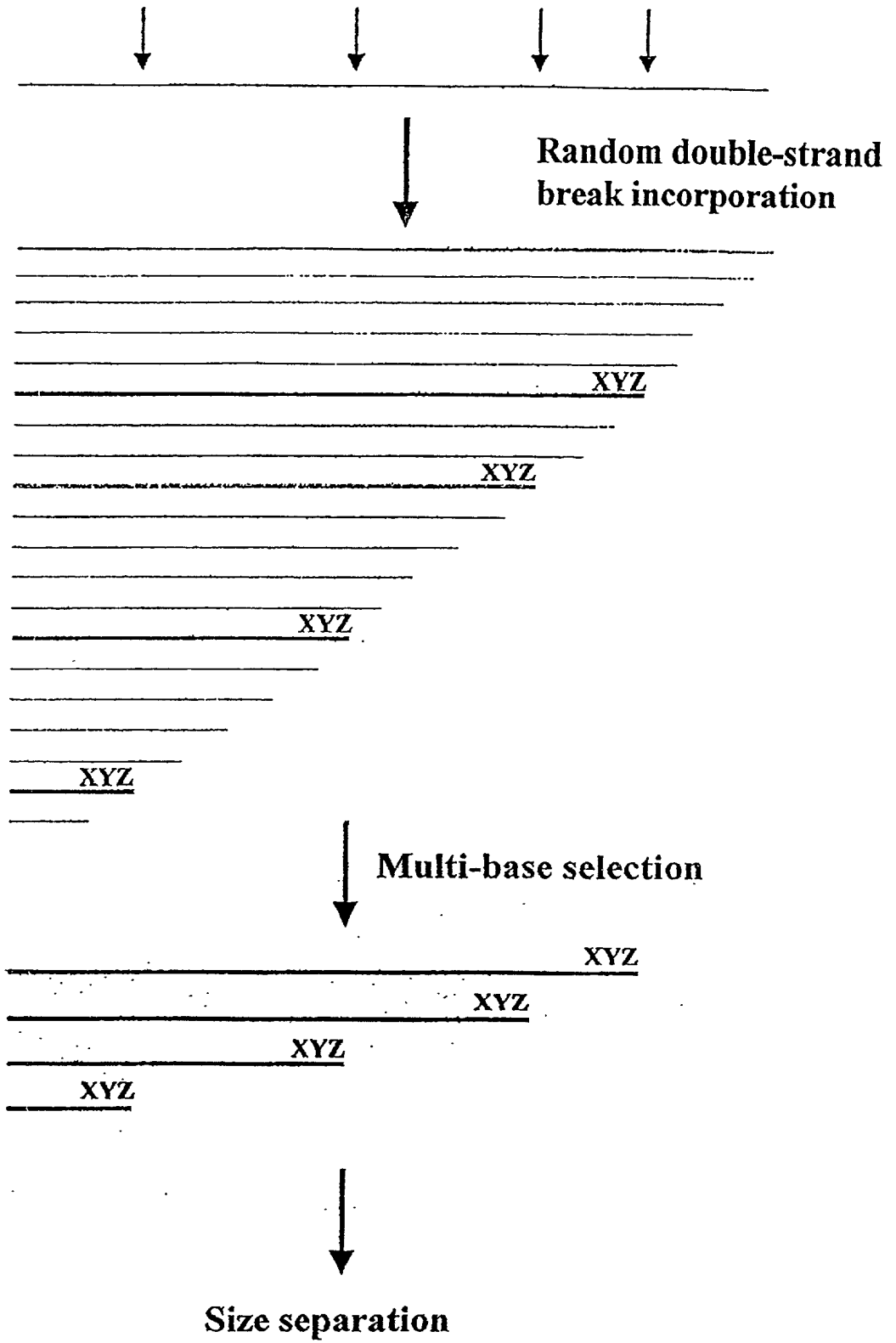


FIG. 31

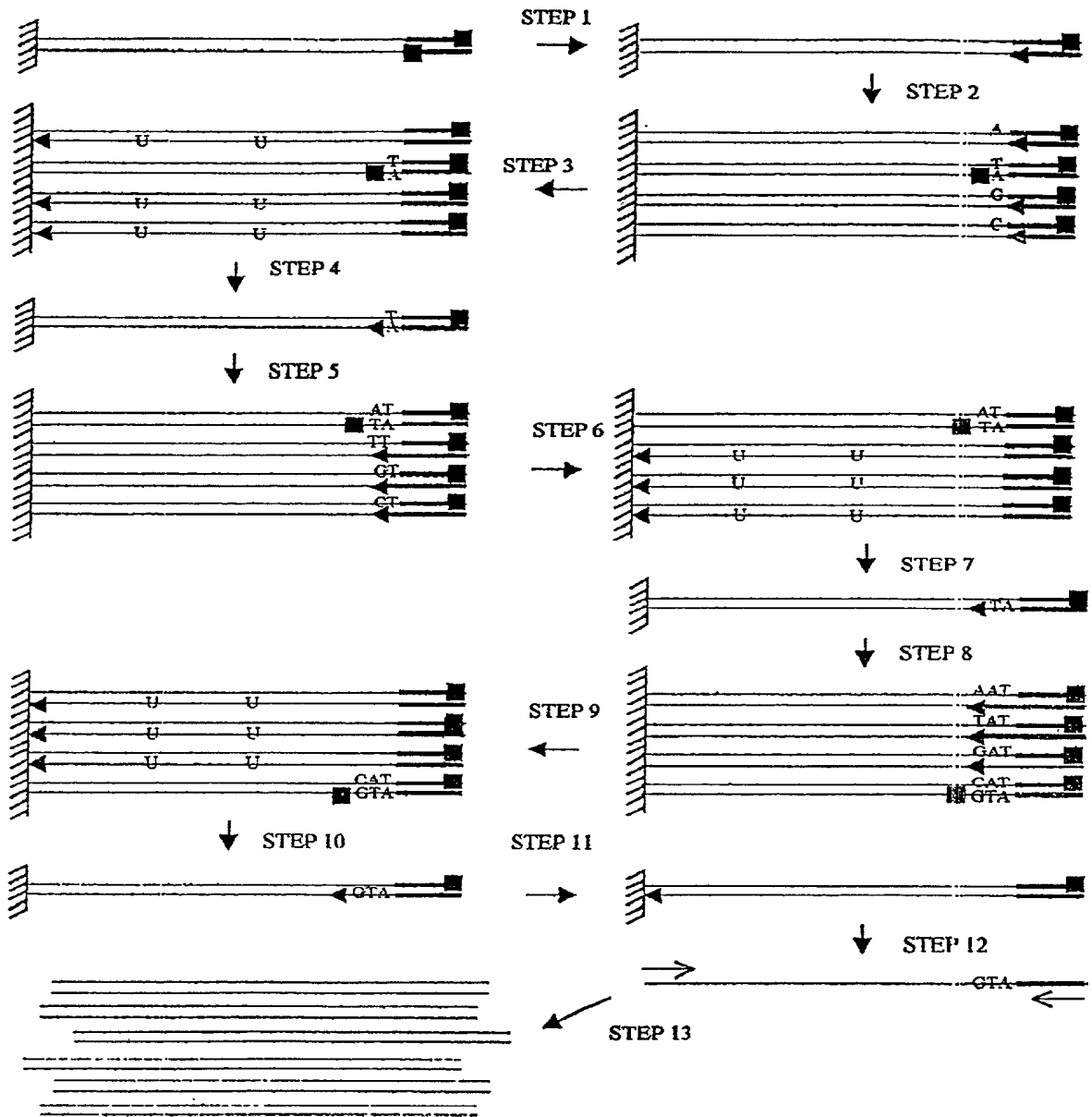
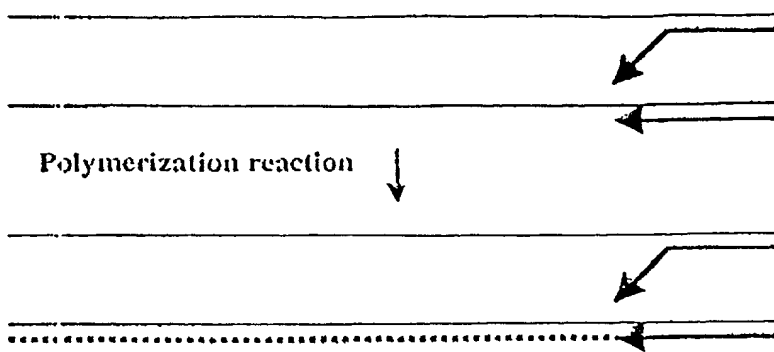


FIG. 32



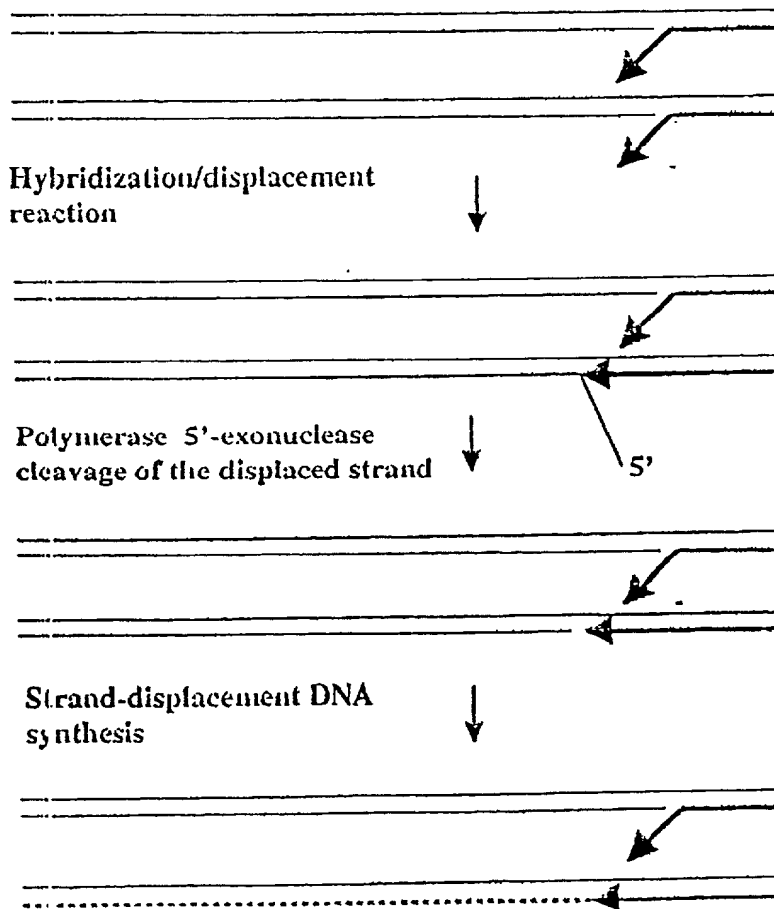
Mis-matched primer

Matched primer

Mis-matched primer

Matched primer

FIG. 33A



Mis-matched primer

Matched primer

Mis-matched primer

Matched primer

Mis-matched primer

Matched primer

Mis-matched primer

Matched primer

FIG. 33B